# RESEARCH NOTE

## Antimicrobial susceptibility of 136 *Escherichia coli* isolates from cases of neonatal meningitis and relationship with virulence

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### ABSTRACT

The susceptibility of 136 *Escherichia coli* isolates from cases of neonatal meningitis to amoxycillin, ceftriaxone, nalidixic acid, ciprofloxacin and gentamicin was determined in relation to the carriage of virulence factors and phylogenetic group. Only amoxycillin and nalidixic acid resistance was observed (40% and 3%, respectively). Nalidixic acid resistance alone was associated with non-virulent phylogenetic group A (50% vs. 6% of susceptible isolates; p 0.03). No difference in virulence was observed between two representative nalidixic acid-susceptible virulent group B2 isolates and their nalidixic acid-resistant derivatives in a rat model of neonatal meningitis, suggesting that nalidixic acid resistance does not affect the virulence of *E. coli* strains causing meningitis.

**Keywords** Antimicrobial resistance, *Escherichia coli*, neonatal meningitis, phylogenetic group, rat model, virulence

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*Escherichia coli* is a major cause of meningitis in neonates, and is associated with high rates of mortality and morbidity [1]. Rapid diagnosis and appropriate empirical antibacterial chemotherapy are essential for successful management. The high frequency of resistance in *E. coli* to aminopenicillins means that third-generation cephalosporins are currently considered to be the drugs of choice for treatment of *E. coli* meningitis [2]. Fluoroquinolones, although not licensed for use in infants, have proven to be safe and effective in this setting [3]. The antibiotic susceptibility profiles of *E. coli* isolates from neonates with meningitis are poorly documented [4,5]. There is some evidence that resistant *E. coli* strains may be less virulent than susceptible strains, but it is not known whether this also applies to isolates from cases of neonatal meningitis [6–8].

The present study determined the susceptibilities of 136 *E. coli* isolates, referred to the French National *E. coli* Reference Centre between 1995 and 2005, from the cerebrospinal fluid of neonates with meningitis throughout France. Susceptibility to nalidixic acid was tested by the disk-diffusion method, and MICs of amoxycillin, ceftriaxone, ciprofloxacin and gentamicin were determined using the agar dilution method with CLSI interpretative criteria [9]. The phylogenetic group of all the isolates and the presence of eight putative virulence factor genes characteristic of extra-intestinal pathogenic *E. coli* strains were determined as described previously [10].

Nalidixic acid-resistant mutants of two archetypal group B2 *E. coli* strains, S95 (O45:K1:H7) from the French collection studied, and C5 (O18:K1:H7) from the USA [10], were obtained by inoculating dense suspensions ($10^9$ CFU/mL) of each test strain on agar plates containing nalidixic acid 16 mg/L. After incubation for 24 h, five colonies from each plate were selected and their ciprofloxacin MICs were determined. To determine the genetic mechanism of resistance, the *gyrA* and *parC* genes were amplified from boiled lysates and sequenced as described previously [11,12]. Two *gyrA* mutants (S95gyrA and C5gyrA) with ciprofloxacin MICs of 0.25 mg/L were then tested in a neonatal rat model of bacteraemia and meningitis [13]. Data were expressed as means ± SD and were compared using a two-sample paired *t*-test. Groupwise comparisons of proportions were based on Pearson's chi-squared test or Fisher's exact test, as appropriate, with $p < 0.05$ considered to be statistically significant.

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**Table 1.** Antibiotic susceptibility, phylogenetic group and virulence factors of 136 *Escherichia coli* isolates from cases of neonatal meningitis

| Variables | No. of isolates (%) | Antibiotic susceptibility | | | |
|---|---|---|---|---|---|
| | | Nalidixic acid | | Amoxycillin | |
| | | Resistant $n$ = 4 (%) | Susceptible $n$ = 132 (%) | Resistant $n$ = 54 (%) | Susceptible $n$ = 82 (%) |
| Phylogenetic group | | | | | |
| B2 | 108 (79) | 2 (50) | 106 (80) | 45 (83.5) | 63 (77) |
| D | 17 (13) | | 17 (13) | 5 (9) | 12 (15) |
| A | 10 (7) | 2 (50)[a] | 8 (6)[a] | 4 (7.5) | 6 (7) |
| B1 | 1 (1) | | 1 (1) | | 1 (1) |
| Virulence factors[b] | | | | | |
| Antigen K1[c] | 125 (92) | 3 (75) | 122 (92) | 49 (91) | 76 (93) |
| *papGII* | 63 (46) | 1 (25) | 62 (47) | 27 (50) | 36 (44) |
| *papC* | 71 (52) | 1 (25) | 70 (53) | 30 (55) | 41 (50) |
| *sfa*/foc[c] | 49 (36) | 2 (50) | 47 (36) | 17 (31) | 32 (39) |
| *sfaS*[c] | 43 (32) | 2 (50) | 41 (31) | 15 (28) | 28 (34) |
| *ibeA*[c] | 53 (39) | 2 (50) | 51 (39) | 20 (37) | 33 (40) |
| *hly* | 18 (13) | 2 (50) | 16 (12) | 12 (22) | 6 (7) |
| *iroN*[c] | 104 (76) | 2 (50) | 102 (77) | 44 (81) | 60 (73) |
| *iucC* | 120 (88) | 4 (100) | 116 (88) | 45 (83) | 75 (91) |

[a]p 0.03.
[b]*papGII*, adhesin PapG class II; *papC*, P fimbriae; *sfa*/foc, S fimbriae; *sfaS*, adhesin S; *ibeA*, invasin; *hly*, haemolysin; *iroN*, iron-uptake system salmochelin; *iucC*, iron-uptake system aerobactin.
[c]Virulence factors specifically involved in meningo-virulence.

The prevalence of resistance to amoxycillin and nalidixic acid was 40% and 3%, respectively. None of the isolates was resistant to ceftriaxone, ciprofloxacin or gentamicin, with $MIC_{90}$ values of 0.064 mg/L and 0.032 mg/L for ceftriaxone and ciprofloxacin, respectively. However, the ceftriaxone MIC for one isolate and the ciprofloxacin MIC for four nalidixic acid-resistant isolates were > ten-fold higher than the respective $MIC_{90}$ values (2 and 0.5 mg/L, respectively). The four nalidixic acid-resistant isolates had a single amino-acid change at Ser83 in GyrA and no alterations in ParC (data not shown). No difference was observed between amoxycillin-resistant and -susceptible isolates. Nalidixic acid resistance was associated with phylogenetic group A (50% vs. 6% of susceptible isolates; p 0.03) (Table 1). No difference in the prevalence of virulence factors between resistant and susceptible isolates was observed.

The virulence of the two archetypal group B2 strains, S95 (O45:K1:H7) and C5 (O18:K1:H7), was assessed in comparison with their nalidixic acid-resistant derivatives in an experimental model of neonatal meningitis. The resistant mutants had a single amino-acid change at Ser83 in GyrA, which is the most frequent mutation involved in quinolone resistance [12]. However, no differences in terms of the level of bacteraemia or the frequency of meningitis were observed between the nalidixic acid-resistant and nalidixic acid-susceptible strains (Table 2).

**Table 2.** Comparison of the virulence of nalidixic acid-susceptible group B2 strains of *Escherichia coli* with their nalidixic acid-resistant derivatives in a neonatal model of meningitis

| Strain | Relevant property | No. of animals | Mean bacteraemia in log CFU/mL (SD) | % of animals with culture-positive CSF |
|---|---|---|---|---|
| C5 | Wild-type | 40 | 5.3 (1) | 31 |
| C5gyrA | *gyrA* mutation | 20 | 5.3 (0.7)[a] | 55 |
| S95 | Wild-type | 18 | 6.4 (1.1) | 39 |
| S95gyrA | *gyrA* mutation | 18 | 6.2 (1.2)[a] | 44 |

CSF, cerebrospinal fluid.
[a]p >0.05 compared to wild-type.

The prevalence of virulence factors and the phylogenetic group distribution of the *E. coli* isolates from cerebrospinal fluid were similar to those reported previously [10]. Although several studies have suggested that resistance to antibiotics, particularly quinolones, is associated with a lower rate and/or reduced level of expression of certain virulence factors by extra-intestinal pathogenic *E. coli* strains [7,8,14], the distribution of the major phylogenetic groups and virulence factors was similar for the amoxycillin-susceptible and -resistant isolates Although the prevalence of nalidixic acid resistance was low, group A was represented significantly more frequently among nalidixic acid-resistant than among nalidixic acid-susceptible isolates. Similarly, nalidixic acid resistance among *E. coli* isolates from adults with urosepsis has been associated with group A [15,16]. Such a relationship could arise from an enhanced exposure of group A strains, which belong mostly to the faecal flora [17], to antibiotics.

Another possible explanation for a higher frequency of nalidixic acid resistance among isolates belonging to less virulent phylogenetic groups, such as group A, would be an incompatibility between quinolone resistance and virulence because of physical loss of virulence factor genes. By activating the SOS response to inhibition of DNA replication, quinolones may contribute to the excision of bacteriophage or virulence genes from the bacterial chromosome. Soto *et al.* [18] reported that sub-inhibitory concentrations of quinolones induced total or partial loss of a pathogenicity island [18], although Johnson *et al.* [19] failed to confirm these results. Horcajada *et al.* [14] found that group B2 quinolone-resistant uropathogenic *E. coli* strains harboured fewer virulence factors than their quinolone-susceptible counterparts, but this could not be confirmed in the present study because of the low prevalence of nalidixic acid resistance among the B2 isolates. Alternatively, DNA gyrase mutations might negatively regulate virulence factor expression [20].

In the present study, the antibiotic resistance profiles of the four isolates that were highly resistant to nalidixic acid and susceptible to ciprofloxacin were associated with alterations in GyrA. However, when the virulence of two archetypal group B2 strains, namely S95 (O45:K1:H7) and C5 (O18:K1:H7), and their nalidixic acid-resistant *gyrA* mutants with a single amino-acid change at Ser83 was determined, the resistant derivatives exhibited the same virulence factors as their susceptible parents (data not shown). The level of bacteraemia and the frequency of meningitis did not differ significantly between the wild-type strains and their resistant derivatives (Table 2), indicating that this particular gyrase mutation, causing the most frequent type of quinolone resistance in *E. coli* [12], does not affect virulence and had no major biological cost, at least in the meningitis model used in this study.

In conclusion, the low prevalence of nalidixic acid resistance observed among the *E. coli* isolates causing neonatal meningitis was in line with the reported association between low virulence and quinolone resistance. However, the experimental data from the present study do not support the hypothesis of a deleterious effect of nalidixic acid resistance on the virulence of *E. coli* strains causing meningitis. Further comparisons between nalidixic acid-susceptible virulent strains and their resistant mutants are needed to reinforce this observation.

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# RESEARCH NOTE

## Molecular, epidemiological and infectivity characterisation of a *Mycobacterium tuberculosis* strain prevalent in Madrid

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**ABSTRACT**

The most prevalent strain of *Mycobacterium tuberculosis* in Madrid, Spain (strain 5) was recovered from 45 cases between 1997 and 2004 and showed a highly homogeneous genetic composition. This strain was not exclusive to Spain, and its spoligotyping signature (ST20) was found in entries from different countries in the SITVIT1 database. Patients infected with strain 5 were more frequently positive for human immunodeficiency virus and autochthonous, and had been in prison more frequently, but strain 5 did not show increased infectivity in an in-vitro model of infection.

**Keywords** Epidemiology, infectivity, *Mycobacterium tuberculosis*, spoligotyping, ST20

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Molecular tools allow different *Mycobacterium tuberculosis* (MTB) strains circulating in a population to be distinguished, with fingerprint data obtained in molecular epidemiology programmes [1] making it possible to identify prevalent strains or genetic families that are over-represented in certain settings [2,3]. In Spain, there has been a sharp increase in the number of cases of tuberculosis among immigrants [4]. It is assumed that some of these patients import MTB strains from their countries of origin, and that such strains could have an impact on the profile of strains circulating in the host population, considering the high rate of transmission between the autochthonous and immigrant populations in this area [4].

Molecular fingerprinting tools have been used in Madrid, Spain since 1997 [5]. The MTB isolates from nine urban districts (1 459 232 inhabitants) in Madrid were genotyped. Isolates from all nine districts were genotyped during 2002–2004, and isolates from five districts during 1997–2001. In total, 1207 MTB isolates were analysed by IS*6110* restriction fragment length polymorphism [6], yielding 867 different genotypes, with 455 (37.7%) isolates grouped in 115 clusters; 51.3% of the clusters included two isolates, and only 9.6% of the clusters included more than six isolates. Nevertheless, some large clusters were detected, with the four largest clusters corresponding to strains 5 (45 cases), 2, 8 and 38 (20 cases each). Strain 5 was